ORIGINAL ARTICLE

DETECTION OF NUCLEAR APPENDAGE IN PERIPHERAL BLOOD NEUTROPHIL LEUKOCYTES FOR SEX DETERMINATION.

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Abstract

Background: Sex determination can be done by the detection of drumstick-shaped nuclear appendage that is present in neutrophil leukocytes from peripheral blood smear of female subjects. **Objectives:** This study was conducted to identify a drumstick-shaped nuclear appendage on neutrophil leucocytes from peripheral blood smear and to analyse the sensitivity, specificity, positive predictive value and negative predictive value of the test in gender determination. **Materials and Methods:** A total of 110 randomly selected blood smears (from 40 male and 70 female subjects) with age range from 19-22 years, were stained with Jenner-Giemsa stain. At least 100 well-stained neutrophils per slide were double-blindly studied by the observers.
Results: Sixty-six out of 70 female subjects and 36 out of 40 male subjects were correctly identified**.** There was 4 false positive result on the male subjects and 4 false negative results on the female subjects in the detection of nuclear appendage. A 94.28% sensitivity, 90% specificity, 94.28% positive predictive value and 90% negative predictive value for the study was calculated. **Conclusions:** The identification and detection of nuclear appendage from neutrophil leukocyte is an easily applicable method that is useful as a screening method in sex determination. Overall, the method also has a high sensitivity, specificity and predictive values.

Keywords: *Neutrophil leukocytes; Nuclear appendages; Sex determination*

Introduction

The first researchers who had demonstrated a morphological sex difference on the nuclei from the cells of the nervous system of mammals were Barr and Bertram in 1949 [1]. It was followed by an extension of the observation to human skin and the method was applied to determine the genetic sex in hermaphrodites[2]. The evidence of structural difference between the nuclei of the neutrophils in the two sexes was then reported by Davidson and Smith in 1954 [3]. Hence, it was also known as "Davidson's bodies". It was reported that these dumbbell-shaped nuclear appendages strongly correlated with female subject and is useful in sex determination. Three percent of neutrophils leukocytes from normal females display a nuclear appendage as a drumstick-shaped mass of dense chromatin, which is 1.5 µm in size. It is attached to one of the nuclear lobes by a thread-like neck to the rest of the nucleus. The presence of nuclear appendage in the female leukocytes represent major part of the XX chromosome mass in female. In contrast, the absence of nuclear appendage in the neutrophil leukocytes can be taken to indicate that there are fewer than two X chromosomes in the cell and this can represent them as males. Sex chromatin is derived from one of the two X chromosomes in the female which replicates its deoxyribonucleic acid much later than the other and is thus positively heteropyknotic[4,5]. Observation on morphology and comparison between 2 different stains: Leishman's and Field presented a moderate agreement in gender determination[6].

This study was conducted to identify only a drumstick-shaped nuclear appendage on neutrophil leucocytes from peripheral blood smear using Jenner Giemsa stain and to analyse the sensitivity, specificity, positive predictive value and negative predictive value of the test in gender determination.

Materials and Methods

This research work was conducted with the permission from the Institutional Ethical Committee. Subjects were medical student volunteers from the University Kuala Lumpur – Royal College of Medicine Perak (UniKL RCMP). Out of 259 students, a convenient random sampling selection was chosen and a total of 110 subjects with age range from 19-22 years were selected which included 40 male students and 70 female students. Ethical issues were considered by anonymizing the collected data, slides were labeled in code number and the detail particulars were not revealed. Letter of consent was given to each subject and the research was conducted only with the permission from the subjects. Venous blood samples were collected into potassium EDTA tubes and thin-blood film were prepared. The blood smears were stained with Jenner-Giemsa method. Duplicate films were prepared from each subject. Only a very small number of films which were technically unsuitable were rejected. The blood smears were examined under light microscope on oil immersion lens after scanning. A double-blind method of study was applied by keeping unknown the observer about the sex of the subject until after examination of the smear. A brief preliminary pilot observation was performed to be familiar in identification of the nuclear appendages from the randomly selected blood films, before a proper observation was performed. Each person independently counted 100 well-stained neutrophils for each slide, from the tail-end of the smears. Detecting the typical drumstick nuclear appendage was regarded as positive or female subject and without detection of the nuclear appendage was regarded as negative or male subject.

Exclusion Criteria: subject who refused to participate in this research.

Statistical analysis: Data were collected and plotted on two-by-two table. The test results were expressed in percentage sensitivity, specificity, positive predictive value and negative predictive value [7].

Results

Peripheral blood collected from 40 males and 70 female subjects, with age range between 20-24 years were observed for sexual differences. Female subjects with identifiable drumstick nuclear appendages were categorized under true positive. Female subjects without being able to identify drumstick nuclear appendages were categorized under false negative. Male subjects without drumstick nuclear appendages were categorised under true negative, and male subjects wrongly identified to have drumstick nuclear appendages were categorised as false positive. A drumstick morphology of the nuclear appendage is identified as a round head and a thin stem (Fig.1). No other non-specific morphologies were counted in as positive nuclear appendage. Figure 2 is a histogram showing total number of subjects detected and not detected for the nuclear appendage in the neutrophil.

A correct identification of sex was made in 36 out of 40 blood smears from the male subjects and 66 out of 70 blood smears from the female subjects. The sex was erroneously observed as male for 4 female subjects and 4 females as male subjects. Table 1 shows the total number and percentage of drumstick positive and negative female and male subjects of the study group. A 94.28% sensitivity, 90% specificity, 94.28% positive predictive value and 90% negative predictive value of the subjects from the study group were calculated as shown in Table 2.

Discussion

Typical nuclear appendages are identified as 'drumstick' that are found in the neutrophils. Identification and detection of the nuclear appendage helps confirm a female subject and absence of nuclear appendage is considered as male subject. Our study demonstrated a high sensitivity (94.28%) and specificity (90%) of the test as well as high positive predictive value (94.28%) and negative predictive value (90%). A recent study done by Tupakula S et al in 2014 was performed on counting in other non-specific appendages such as minor lobes, racket structures and small clubs that were observed in males and that sessile nodules were found only in females. They had demonstrated that the incidence of drumstick neutrophil nuclear appendage is a valuable data for sex differences[4]. Another recent study had mentioned that drumstick appearance is the true morphology found out in an average of 5.4 in female and 2.1 in male subjects, whereas the non-specific forms sessile nodules average 0.6 in females and 0.3 in males and the tag and hook forms were 2.9 in females and 7.4 in males. They had reported that it is essential to consider all forms of neutrophilic nuclear appendages for morphological sex differentiation[8]. Chatterjee et al in 2014 found drumstick as a predominant feature in identification of cytological sex[5]. Moreover, a recent study done by Zoja and Rita in 2018 had mentioned that only drumstick appendage is sex-specific and considered for sex diagnosis and drumsticks are significantly higher in females than in males [9]. Hence, we decided to focus only on typical drumstick appendages rather than other non-specific morphologies. We believe this could be the false positive findings of the drumstick in the male subjects in our study. Nodules with a short thick neck and sessile nodules, although nearly diagnostic of the female neutrophils, are more difficult to distinguish from other nodules found in both sexes and can result in more false negative and false positive findings. It might also be due to technical error and intra or inter observer variation.

Brahimi et al had mentioned that blood smear is a reliable tool to determine gender as well as in determining the hormonal influence on the subject. Neutrophils bear the same amount of appendages in both gender, but the number of drumstick form is significantly greater in females and the increase in the number of tag form appendages is significant in male, and that this might be a manifestation of a high androgen level in men [10]. Thus, more information could be obtained if we performed our study by further counting in other non-specific morphological features on the nuclear appendage and the hormonal status of the subject.

Other than neutrophil nuclear appendages, studies have also shown that identification of buccal Barr bodies and cells from the dental pulp could be applied as reliable parameters in medico-legal cases for sex determination [8, 11, 12, 13, 14]. Further comparative study on the significant value of sex determination using neutrophil nuclear appendages and Barr body identification will be of great value. The usefulness of drumstick nuclear appendage in chronic myeloid leukaemia was traced back since 1961 by Tomonaga et al, and recently, continuation studies on myeloproliferative disorder and leukaemia has found out much support on further anti-leukemic therapy [15-17].

Recently, Selvi et al in 2018 have mentioned that Davidson bodies in blood smear are highly specific when compared to Barr bodies in buccal smear. Morphological gender determination using Davidson bodies in haemato-pathology is easy, reliable, less time consuming and cost effective[19]. The identification of nuclear appendage in sex detection can be used as a mass screening test as it is easy to perform, able to obtain immediate result and cost-effective, although it may not be a confirmatory diagnostic test. Recent advances in technology aids in identifying X chromosome-specific nucleic acid probe confirms the position of the X chromosomes in the drumstick structure of leukocyte nuclei by *in situ* hybridization are considered confirmatory methods for sex determination. The demonstration of human chromosome abnormalities, and the determination of sex linked disorder can also be done by using this simple technique [12, 18].

Conclusion

Based on our findings, higher percentage of female subjects are found to have presence of nuclear appendage in neutrophil while higher percentage of male subject are found to have absence of nuclear appendage in neutrophil. Thus, there is evidence of a significant association between gender of the persons and the presence or absence of nuclear appendage in neutrophil leukocytes. It is a simple test that can be performed with little training and experience and therefore is a suitable cost effective test as a screening test to perform in remote areas where sophisticated and expensive facilities are not available. However, we believe that a further confirmatory test such as karyotyping should be performed in critical and specific situation such as medico-legal cases and ambiguity in sex.

Acknowledgement

We acknowledge our year 2 medical students and staff from our teaching laboratory in UniKL-RCMP who had cooperatively participated in this research work in the Special Study Module.

Conflict of interests

The authors declare no conflict of interests for the publication of this article.

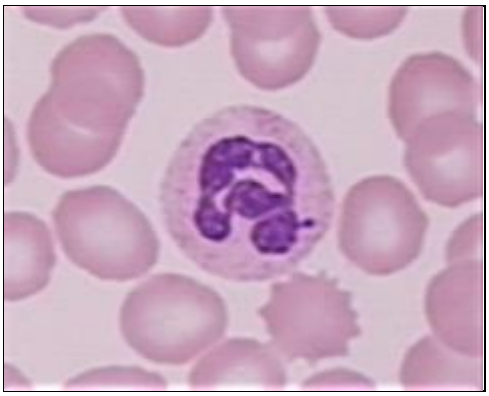

Figure 1. A typical drumstick nuclear appendage identified in the peripheral blood smear of a female subject.

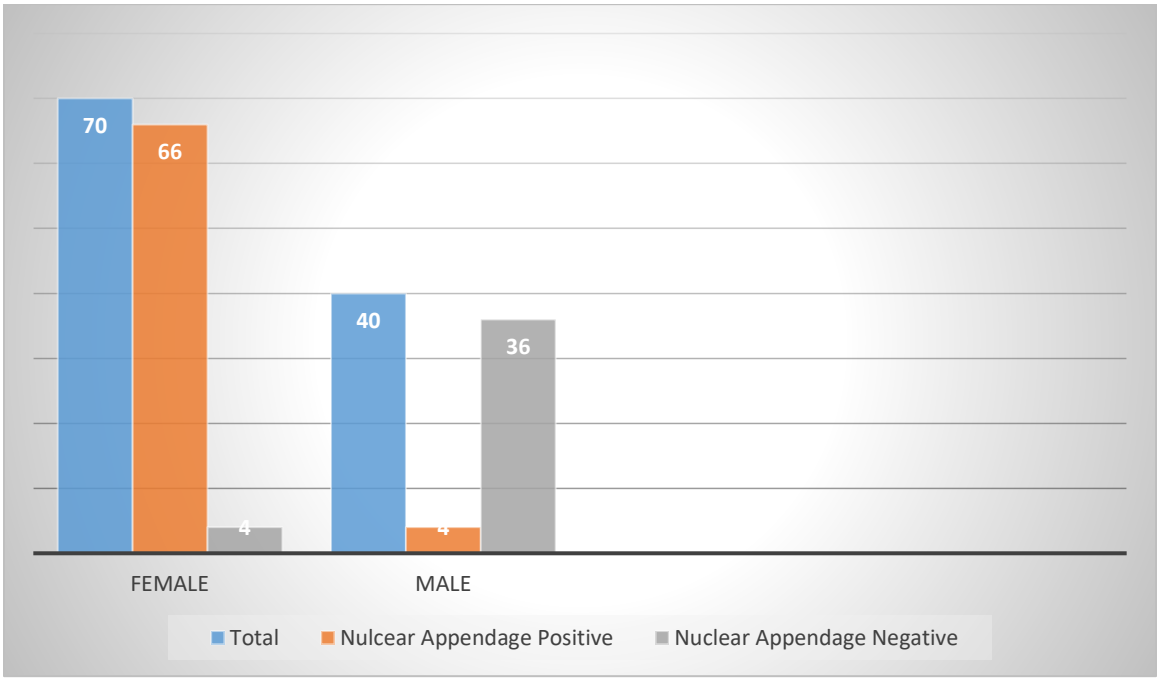

Figure 2. Histogram showing total number of subjects detected and not detected for nuclear appendage

Table 1. Total number (n) and percentage (%) of drumstick positive and negative female and male subjects of the study group.

	Drumstick Positive		Drumstick Negative	
Sex (n)	**n**	**%**	**n**	**%**
Male (40)	4	3.64	36	32.73
Female (70)	66	60	4	3.64

Table 2. Sensitivity, Specificity, Positive Predictive Value and Negative Predictive Value of sex determination by the detection of nuclear appendage in the subjects from the study group.

Total Number of Subjects (n-110)			
Sensitivity	**Specificity**	**Positive Predictive Value**	**Negative Predictive Value**
94.28%	90.0%	94.28%	90.0%

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